MICROBIOLOGICAL DECOMPOSITION OF BEECH LITTER*

Toshi SAITÔ

THE decomposition of fallen leaves under natural conditions has been the subject of much investigation. To understand the process of decomposition, it is essential to have a clear concept of the microbial population in litter and the processes that the various representatives of the population bring about, as well as the environmental conditions modifying the nature of the population and its activities.

The present paper gives an account of the microbial population in the litter of a beechwood and its microbiological decomposition on the south-facing slope of Mount Hakkôda in Aomori Prefecture—the northernmost prefecture of Japanese Main Island.

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ACCUMULATION AND TRANSFORMATION OF FALLEN LEAVES

The area under investigation is situated in the neighbourhood of Tsuta hot spring at an altitude of 470 m. above sea level. The vegetation there is dominated by *Fagus crenata*, up to 30 m. high, mixed with scattered *Quercus crispula*. The under tree-layer is poor, consisting mainly of *Fagus crenata*, *Lindera umbellata*, *Acer japonicum* and *Viburnum furcatum*. And the field layer is almost open.

The profile through the so-called L-, F- and H-layers may be divided into the following six horizons of varying thickness undergoing different degrees of decomposition, although the depth of deposits varies a great deal from place to place:

I-layer: The uppermost layer, i.e. L-layer, consisting of brown fallen leaves not yet subject to decomposition and only slightly altered leaves. Though the moisture of this layer varies considerably with the weather, the leaves are liable to dry up rapidly and thoroughly. 0.5–2.0 cm. in depth.

II-layer: In this layer, each of brown leaves changes partly to yellowish, sometimes to whitish yellow. These leaves are still hard and retain their own structure. 0.5–1.5 cm. in depth.

III-layer: Fungus mycelia are wide-spread in abundance in this layer, and the mouldy yellowish leaves become thinner gradually. But the structure of leaves is

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generally well preserved. 0.5–2.0 cm. in depth.

IV-layer: Fibrous decomposed leaves, very often fragmentary, of various stages of decomposition are mixed with a large amount of undecomposed grayish brown leaves, which are fragmentary but still retain the structure. Both kinds of leaves are generally coarser in texture than in the underlying layer. 0.5–1.0 cm. in depth.

V-layer: The features of this layer are on the whole similar to IV-layer, but decomposition advances more, the leaves being broken into powder when strongly crushed by hand. 0.5–1.5 cm. in depth.

The layers II to V correspond to F-layer. ??

VI-layer: This consists for the most part of much decomposed, amorphous organic matter and contains abundant rootlets. It corresponds to H-layer. 1.0–1.5 cm. in depth.

The attack on the brown leaves of fungus mycelia, mostly of basidiomycetes whose hyphae can be identified based on the presence of clamp connections, is likely to lead to changes of colour as a result of digestion of tannins. This action seems to be the first step of decomposition proceeding in II- and III-layers. Generally, the degree of decomposition is various even in the same layer, and, furthermore, the decomposition takes place not always uniformly on the whole surface of a leaf. It is not seldom that, in IV- and V-layers, fragmentary brownish leaves without attack of fungi are seen mixed with residues resulting from the attack of fungi. Thus these brownish leaves having got rid of the fungal attack may undergo decomposition in a different manner in comparison with II- and III-layers where basidiomycetes are in the main responsible for the decomposition.

Coefficients of weight and of decomposition

In order to know the degree of decomposition, two coefficients are calculated with each of brown, yellowish and thinner yellowish leaves. The coefficient of weight is the dry weight in gram per square decimetre of a leaf and the coefficient of decomposition is the ratio of the difference between the coefficient of weight of the original brown leaf and the coefficient of weight of the leaf under decomposition to the former. Since the thickness of leaves is highly variable ranging from *c.* 70 μ to *c.* 150 μ, the coefficients vary a great deal. Therefore, leaf samples are distinguished in three ranks: "thick leaves" are more than 130 μ in thickness, "thin leaves" less than 100 μ, and "medium leaves" between 100 μ and 130 μ. The values plotted in Fig. 1 are the average of ten leaves, each of which is partly brown, yellowish and thinner yellowish.

As will be seen from Fig. 1, the rate of changes in "thick leaves" is larger than that of the "medium" and "thin". This seems to be due to the consumption of a

larger amount of decomposable substances in the tissue of "thick leaves".

MICROBIOLOGICAL ANALYSIS

The samples were taken on August 15, 1955, from ten spots in each layer.

Since it was desired to compare the numbers of microbes, the plate method was bused in spite of its serious limitation, by the use of albumen agar for bacteria and actinomycetes and of acid glucose peptone agar for fungi. For cellulose-decomposing bacteria, were used DUBOS' solution for the aerobes and OMELIANSKI's solution for the anaerobes (see FRED and WAKSMAN, 1928). The numbers of various microbes, together with the moisture and the pH value, are recorded in Table 1. The volume in the litter itself per gram of dry meterials from different layers varies largely, especially a marked difference being noticed between VI-layer and the other layers, so that

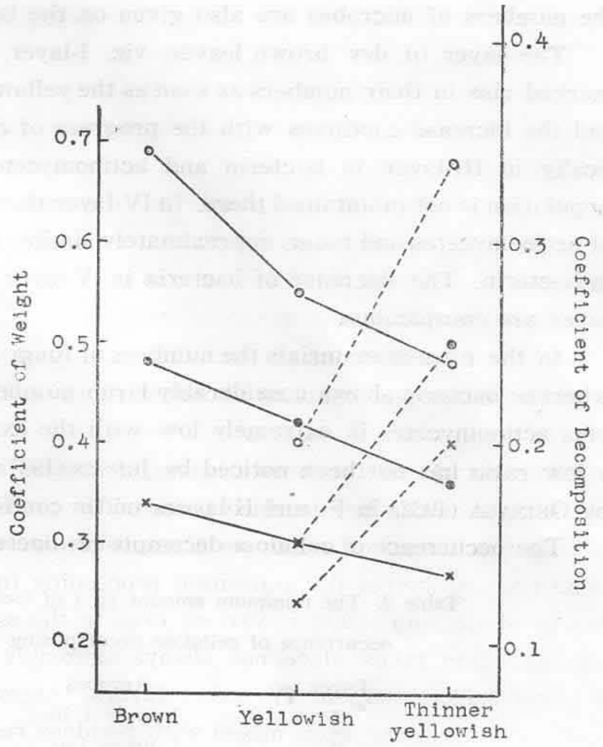

Fig. 1. The coefficient of weight and the coefficient of decomposition (dotted lines). Circles, "thick leaves". Points, "medium leaves". Crosses, "thin leaves".

Table 1. Moisture, pH values and the plate counts of various microbes ($\times 10^3$) per g. of dry material and per cm.^{3} of deposits (in parentheses).

Layer	Moisture %	pH	Bacteria (B)	Actinomycetes (A)	Fungi (F)	F/B+A×100
I	19	5.1	2030 (26)	16 (0.2)	18 (0.2)	0.880
II	40	5.0	60000 (1020)	27 (0.4)	40 (0.7)	0.067
III	44	5.0	257000 (6680)	71 (1.8)	22 (0.5)	0.009
IV	45	5.2	225000 (6750)	82 (3.3)	30 (1.2)	0.013
V	52	5.1	54000 (2540)	62 (2.9)	14 (0.7)	0.026
VI	45	5.0	9600 (2110)	18 (3.9)	58 (12.0)	0.603

the numbers of microbes are also given on the basis of the volume.

The layer of dry brown leaves, viz. I-layer, is poor in microbes. There is a marked rise in their numbers as soon as the yellowish change of leaves takes place, and the increase continues with the progress of decomposition, culminating practically in III-layer in bacteria and actinomycetes, though the increase of fungal population is not maintained there. In IV-layer there is some increase in the numbers of actinomycetes and fungi, approximately similar numbers to III-layer being counted in bacteria. The decrease of bacteria in V-layer and the increase of fungi in VI-layer are conspicuous.

In the present materials the numbers of fungi and actinomycetes are not large, whereas bacteria shows considerably large numbers. The ratio of fungi to bacteria plus actinomycetes is extremely low with the exception of I- and VI-layers. Such a low ratio has not been noticed by JENSEN (1931) and SAITÔ (1955) in some soils nor by OKINAGA (1952) in F- and H-layers under coniferous forests.

The occurrence of cellulose-decomposing bacteria is shown in Table 2. Aerobic

Table 2. The minimum amount (g.) of fresh material in which the occurrence of cellulose-decomposing bacteria is noticed.

Layer	Aerobes	Anaerobes
I	$1/10$ or $1/10^2$	—
II	$1/10^4$ or less	—
III	$1/10$	— or $1/10$
IV	$1/10^3$	$1/10^2$
V	$1/10^2$	$1/10^2$
VI	$1/10^2$	$1/10$

forms are most abundant in II-layer and fewer in III-layer, while the greatest numbers of anaerobic forms are recorded in IV- and V-layers. Anaerobes thrive only in deeper layers. OKADA (1931, 1935, 1936) reported that both aerobic and anaerobic cellulose-decomposing bacteria are practically absent from or very few in the acid soil of the *Pseudosasa*-association of Mt. Hakkôda.

Basidiomycetes probably play an important role in the transformation of fallen leaves. Nevertheless the plate method gives no information whatever concerning the mycelia of basidiomycetes which attach more or less firmly to the plant debris in the litter. Therefore, the abundance of their mycelia should be estimated by the macro- and microscopic examination of materials. Table 3 shows the

Table 3. The density of basidiomycetes.

Layer	Density
I	—
II	++
III	++++
IV	+
V	+
VI	+

density of the basidiomycetous mycelia thus estimated in each layer. So far as the initial stages of decomposition of beech litter are concerned, it is suggested that basidiomycetes are more active than other microbes.

QUALITATIVE ASPECTS OF THE FUNGAL FLORA

This section is concerned with the distribution of important species of filamentous fungi in different horizons. Some twenty species listed in Table 4 constitute the majority of them, the other species being not identified mostly on account of sterility.

Table 4. The plate counts of fungus species per g of dry material ($\times 10^2$).

Layer :	I	II	III	IV	V	VI
Absidia glauca	8	62	23	193	54	71
Mucor ramannianus	16	10	17	9	18	34
Mucor racemosus	6	11	5			13
Mucor sp.	2	6			5	
Phoma sp.		3				
Trichoderma viride	22	12	23	14	24	54
Trichoderma album	6	3				260
Hyalopus sp.						13
Penicillium lapidosum	30	6	12	7	11	30
Penicillium raistrikii	37	190	12	12	9	13
Penicillium chrysogenum	10	5	7	6	2	13
Penicillium herquei	4	6				
Verticillium terrestre	2					
Spicaria elegans						13
Papularia sp.		2				
Pullularia pullulans	1					
Cladosporium herbarum	10	8	6	5		
Alternaria sp.	7					
Fusarium sp.	2	13				
Yeasts			80	42		

From the table it will be seen that the distribution of different species of fungi is relevant to the progress of decomposition. The genus *Penicillium* is most abundant in I- and II-layers. It extends right through the various horizons and most species of it occur so recurrently and abundantly that they might be classed as the characteristic species: These are *Penicillium lapidosum*, *P. raistrikii* and *P. chrysogenum*. It is interesting to note the occurrence in abundance of yeasts in III-layer, which are absent from the other horizons with the exception of IV-layer. The outstanding features of IV- and V-layers are the dominance of *Absidia glauca*, which occurs constantly in every layer together with *Mucor ramannianus* and *Trichoderma viride*. *Trichoderma album* is encountered in VI-layer in a great number.

SMIT and WIERINGA (1953) reported that *Pullalaria pullulans* is the chief pectin-

splitting organism in the early decomposition of litter. McLENNAN and DUCKER (1954) also isolated this organism from the sparse litter of heath, and moreover found that ascosporic and sclerotial types characterized the litter fungi. According to my results, *Pullularia pullulans* is obtained only from the surface layer in a small number. With regard to the sclerotial forms, *Penicillium lapidosum* and *P. raistrikii* were isolated from my material.

EVOLUTION OF CARBON DIOXIDE

As an index of the rate of decomposition, the production of carbon dioxide was determined with an apparatus after BOYSEN JENSEN (1932). Air freed from carbon dioxide by passage through soda lime and a concentrated solution of potassium hydroxide was circulated over the surface of the samples at a rate of 1/2 litre per hour at room temperatures of 29 to 29.5°C. in summer-time. The results are shown in Table 5.

Table 5. Carbon dioxide evolution (mg.) per hour per 100 g of dry material and per 100 cm^3 of deposits.

Layer	per 100 g.	per 100 $cm.^3$
I	3.0	0.04
II	28.2	0.5
III	63.0	1.6
IV	32.2	1.1
V	21.9	1.1
VI	6.4	1.4

The yield of carbon dioxide is largest in III-layer which is rich in bacteria and basidiomycetes. VI-layer shows an evolution of carbon dioxide to some extent despite the fact that bacteria and basidiomycetes are not abundant in it. The productions of carbon dioxide of IV- and V-layers are approximately equal to each other. The correlation does not always exist between the numbers of micro-organisms and the yield of carbon dioxide.

SUMMARY

1. In the beechwood on the south-facing slope of Mt. Hakkôda, the profile of the litter may be divided into several horizons where the leaves of beech undergo different degrees of decomposition.

2. In the upper horizons overlain by undecomposed brown fallen leaves, individual leaves, turn partly yellowish with a heavy infection with basidiomycetous mycelia associated with a vigorous growth of bacteria, and become thinner without losing their own structure at first.

3. In the lower horizons, where the decomposition advances much more leading

to transformation into amorphous debris, the numbers of bacteria and actinomycetes are reduced, but the plate count of fungi tends to increase.

4. Apart from basidiomycetes mentioned above, filamentous fungi, as well as actinomycetes, are, broadly speaking, by far fewer in comparison with bacteria.

5. A few species of *Penicillium*, *Absidia glauca*, *Mucor ramannianus* and *Trichoderma viride* are wide-spread.

6. Carbon dioxide production is prominent in the horizon which is rich in bacteria and basidiomycetes.

College of Arts and Science, Faculty of Education, Tôhoku University, Sendai.

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